BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 072 111
A1

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 82303789.0

(22) Date of filing: 19.07.82

(51) Int. Cl.³: C 07 F 9/10, C 08 G 65/32, A 61 K 9/66

(30) Priority: 20.07.81 US 284675

(43) Date of publication of application: 16.02.83 Bulletin 83/7

(84) Designated Contracting States: BE CH DE FR GB IT LI NL SE

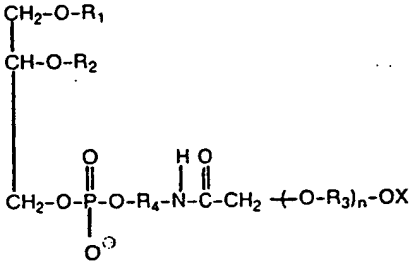
(71) Applicant: LIPID SPECIALITIES, INC., 281, Albany Street, Cambridge Massachusetts 02139 (US)

(72) Inventor: Sears, Barry D., 5 Cleveland Road, Marblehead Massachusetts 01945 (US)

(74) Representative: Myerscough, Philip Boyd et al, J.A.Kemp & Co. 14, South Square Gray's Inn, London, WC1R 5EU (GB)

(54) **Synthetic phospholipid compounds and their preparation.**

(57) Synthetic phospholipid compounds are provided having the structural formula:



where R_1 and R_2 are hydrogen or saturated or unsaturated acyl, R_3 is alkylene, R_4 is C_2–C_{10} alkylene, X is hydrogen or alkyl and n is from 0 to 200. The compounds can be prepared by coupling a phosphatidyl alkanolamine to a carboxylic analog of a polyalkylene oxide.

DESCRIPTION

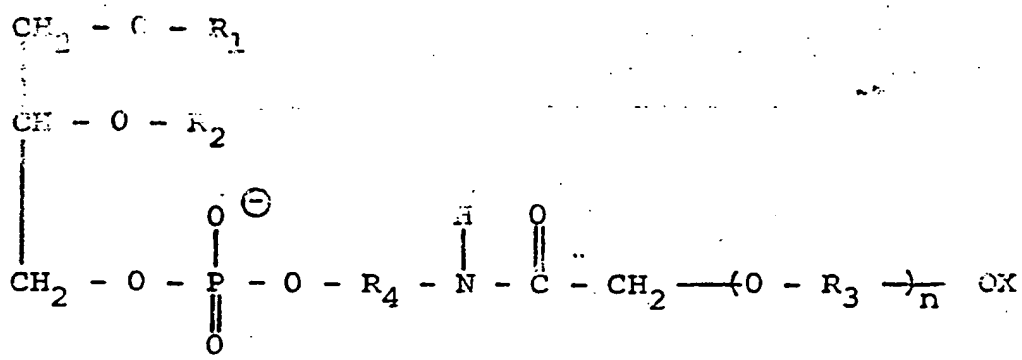
TITLE: "SYNTHETIC PHOSPHOLIPID COMPOUNDS AND THEIR PREPARATION"

Phospholipids, such as lecithin, are amphipathic compounds in that they consist of both hydrophobic and hydrophilic groups or regions within the same molecule. The balance between these hydrophobic and hydrophilic regions determines their physical properties in an aqueous environment. The uses of natural phospholipids as additives are numerous in the food industry (e.g. as emulsifiers), in cosmetics, for industrial uses, and for the pharmaceutical industry, especially in the preparation of drug-delivery systems. U.S. Patents Nos. 4,086,257, 4,097,502, 4,097,503, 4,145,410 and 4,159,988 disclose various modifications of the polar-head-group region of natural phospholipids which lead to unique and unexpected physical properties.

Further, various derivatives of lecithin are known, such as, for example, oxyalkylated lecithin compounds (see U.S. Patents 2,310,679 and 3,085,100), and phosphatidyl-alkanolamine derivatives (see for example U.S. Patents 2,801,255, 3,542,820, 3,577,446 and 4,254,115). It is desirable to provide novel synthetic phospholipids, particularly having enhanced, controlled, solubility properties in an aqueous environment.

This invention relates to novel phospholipid compounds in which the polar-head-group region is modified by the covalent attachment of polyalkylene oxide polymers of various molecular weights, to their preparation and to their use, particularly in an aqueous environment especially their use to encapsulate drugs in a drug-delivery system.

The phospholipid compounds of the invention are phosphatidyl alkylene oxide compounds having the structural formula:



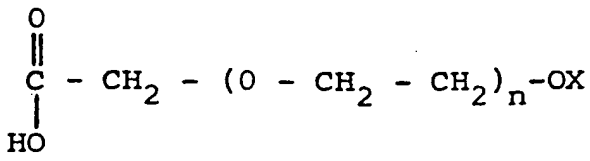
where R_1 and R_2 represent hydrogen or saturated or unsaturated straight-chain or branched-chain acyl groups, R_3 represents an alkylene group, particularly, but not limited to, ethylene, propylene and mixtures thereof, and R_4 represents a C_2-C_{10} alkylene group, particularly a dimethylene group $-CH_2CH_2-$ as in natural lecithin. The number of alkylene oxide groups in the polymer, designated as n, may vary from 0 to 200 or more; e.g. 10 to 100, such as 3 to 20. X is hydrogen or alkyl, such as a C_1-C_4 group like methyl. The attachment of a relatively hydrophilic polyalkylene oxide group, particularly a group derived from polyethylene oxide, alters the hydrophilic to hydrophobic balance within the phospholipid, in order to give unique solubility properties to the phospholipid compound in an aqueous environment.

These novel compounds are quite unlike the compounds described, for example, in U.S. Patents 2,310,679 and 3,085,100, which are products from the coupling of ethylene oxide or similar compounds to crude soy "lecithin". The use of the term "lecithin" describes a number of compounds including lecithin (i.e. phosphatidylcholine), a compound that cannot react with ethylene oxide. On the other hand, soy "lecithin" does contain phosphatidylethanolamine, phosphatidylinositol, and a variety of glycolipids. All of these compounds in crude "lecithin" can react with ethylene oxide or similar compounds containing a reactive cyclo oxide group to form various adducts.

For example, in phosphatidylinositol and with glycolipids, the reactive groups in these molecules are hydroxyl groups which will form an ether linkage when reacted with ethylene oxide. Phosphatidylethanolamine, which contains a primary amino group, will react with ethylene oxide to form an alkylamine linkage (see N. Schonfeldt, "Surface Active Ethylene Oxide Adducts" Pergamon Press, 1969). In both cases, these adducts should not be biologically degradable, and, therefore, such compounds will be undesirable for use in the cosmetic and pharmaceutical industries.

The phospholipids of the invention comprise synthetic phospholipids in which the linkage between the synthetic ethylene oxide or propylene oxide polymer and the naturally occurring phospholipid is a biologically degradable linkage; i.e. an amide linkage, which makes these novel phospholipid compounds useful for cosmetic and pharmaceutical uses.

The preparation of these compounds is best accomplished by the coupling of the appropriate carboxylic analog of the polyalkylene oxide polymer to the phosphatidylalkanolamine molecule, such as the phosphatidyethanolamine molecule. For example, there can be used a polyethylene oxide polymer analog having the structure:



where X is hydrogen or alkyl and where n can vary from 0 to 200. The carboxylic analog of the polyethylene oxide polymer can be prepared by using either $KMnO_4$ or pyridium dichromate or other oxidizing agent, to oxidize a suitable polyalkylene oxide polymer starting material as shown below:

$$HO - CH_2 - CH_2 - (O - CH_2 - CH_2)_n - OX$$

The oxidized compound is then further purified via distillation and ion-exchange chromatography. The carboxylic analog of the polyethylene oxide polymer is activated by a convenient activating agent, such as oxayl chloride or 1,1-carbonyl diimidazole. The activated carboxylic derivative of the parent polyethylene oxide polymer is then coupled to the phosphatidylethanolamine via an amide linkage, to form the phospholipid analog compounds of the invention.

The phosphatidylethanolamine or synthetic analogs of phosphatidylethanolamine can either be isolated from natural sources, synthesized according to established chemical procedures, or enzymatically synthesized using the corresponding phosphatidyl choline compound in the presence of ethanolamine and phospholipase D. R_1 and R_2 can represent straight or branched carbon acyl groups having from 2 to 24 carbon atoms; i.e. C_2-C_{20}, and can be acyl groups from unsaturated fatty acids, such as, but not limited to, oleic, stearic, linoleic, linolenic, palmitic, myristic, or arachidonic acids.

The reaction of the phosphatidylethanolamine and the carboxylic derivative of the polyethylene oxide polymer is carried out in an inert solvent, such as dry benzene. The progress of the reaction can be monitored by thin-layer chromatography. Purification of the final product, if necessary, may be carried out using column chromatography.

In the phospholipid compounds of the invention, the polar head group of the phosphatidylethanolamine has been modified to alter their physical properties, by the inclusion of a polyalkylene oxide group. In all cases where natural phospholipids can be used, such as in drug-delivery systems, in cosmetics, in

food and industrial uses, in treating atherosclerosis, for intravenous nutrition, and other uses, these new synthetic phospholipid compounds can be used alone or in combination with other natural phospholipids, especially phosphatidyl choline. Biologically the synthetic phospholipids will be physiologically inert. For example, polyethylene oxide groups attached to proteins are nonimmunogenic and well tolerated by the body (see Abuchowski et al J. Biol. Chem. 252, pp 3578-3581 (1977)). The covalent linkage between the polyethylene oxide group and the phosphatidyl-ethanolamine is biologically degradable, and phosphatidylethanolamine itself is a natural compound.

As a result, these novel compounds will have great utility in encapsulating drugs as drug-delivery systems that can either be administered orally or via injection, such as in the encapsulation process disclosed in U.S. Patent 4320121, as well as in the method of U.S. Patent 4,016,100.

The presence of the hydrophilic alkylene oxide group, particularly a polyethylene oxide group in these new phospholipids, also gives rise to novel and unexpected physical properties in an aqueous environment. Unsaturated phosphatidylethanolamines, especially those isolated from soy beans, do not form any stable type of structure in water. On the other hand, although gangliosides have a similar hydrophobic region compared to phosphatidylethanolamine, the polar region of the ganglioside molecule is composed of hydrophilic oligosaccharides. The presence of these oligosaccharides allows the ganglioside to organize into a stable micelle upon hydration with water. By covalently attaching a hydrophilic polymer group, such as a polyethylene oxide group, to phosphatidyl-ethanolamine, a phospholipid analog to ganglioside is essentially synthesized. It should also be noted that, while no molecular species of phosphatidylethanolamine

will form a stable structure in an aqueous environment, the phospholipid analog compounds described herein do form stable structures upon hydration.

The actual organization of these structures, however, will depend at least in part on the selected acyl chain composition of the phosphatidylethanolamine and the length of the polyalkylene oxide group. Moreover, the combination of these new phospholipid analogs with natural phospholipids, especially in small sonicated phospholipid vesicles, will stabilize those vesicles which are naturally unstable. This stabilization may occur by the presence of the polyalkylene oxide group which may act as a physical barrier that prevents vesicle-vesicle contact that might result in the subsequent coalescence of the sonicated phospholipid vesicles.

The following Examples will help to illustrate the invention.

Example 1

1120 Micromoles of soy phosphatidylethanolamine were taken to dryness under high vacuum. 4480 Micromoles of monomethyl polyethylene oxide carboxylic derivative (average molecular weight of 134) and 2240 micromoles of 1,1-carbonyl diimidazole were mixed in 5 ml. of dry benzene. The solution was heated to 40°C until the bubbling ceased. This solution was added to the dry phospholipid and the final volume was reduced to 3 ml. and heated for 3 hours at 65°C. Thin-layer chromatography indicated a complete reaction. The product was purified by silicic acid chromatography. The final yield of the purified product was 74%. The product had a R_f of 0.46 in a solvent system composed of 75/25/1 ($CHCl_3$/methanol/NH_4OH). In the same solvent system, phosphatidylethanolamine had an R_f of 0.14.

Example 2

1120 Micromoles of purified soy phosphatidyl-ethanolamine were taken to dryness under high vacuum. 1680 Micromoles of the monomethyl polyethylene oxide carboxylic derivative (average molecular weight 1900) and 1400 micromoles of 1,1-carbonyl diimidazole were dissolved in 10 ml. of dry benzene. The solution was heated at 40°C until the bubbling had ceased. The mixture was then added to the dry phospholipid and the total volume was reduced to 5 ml. and heated for 3 hours at 65°C. The product was purified by silicic acid chromatography to give an overall yield of 7%. The R_f of the product in the same solvent system as in Example 1 was 0.78.

Example 3

800 Micromoles of purified soy phosphatidyl-ethanolamine were taken to dryness under high vacuum. 2400 Micromoles of monomethyl polyethylene oxide carboxylic derivative (average molecular weight 266) were dissolved in 11 ml. of dry benzene, and 2160 micromoles of 1,1-carbonyl diimidazole were added and the solution was heated at 40°C until the bubbling had ceased. The solution was added to the dried phospholipid and the volume was reduced to 3 ml. The reaction was heated at 65°C for 3 hours. The product was purified by silicic acid chromatography to give a yield of 34%. The R_f of the product in the same solvent system as in Example 1 was 0.62.

Example 4

491 Micromoles of soy phosphatidylethanolamine were taken to dryness under high vacuum. 1560 Micromoles of methoxyacetic acid and 1560 μ moles of 1,1-carbonyl diimidazole were dissolved in 10 ml. of dry benzene and heated at 40°C until the bubbling had ceased. The solution was added to the dry phosphatidylethanolamine and the volume was reduced to 3 ml. The solution was heated to 60°C for 3 hours.

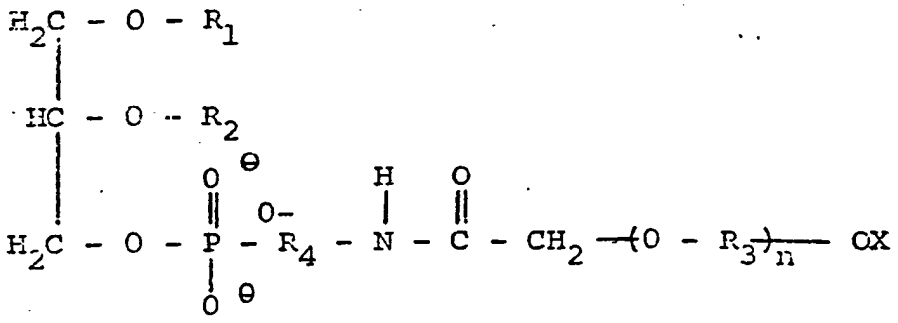
Thin layer chromatography indicated a complete reaction. The product was extracted with a Folch extraction system and the lower phase was taken to dryness. The yield was 92%. The R_f of the compound in the same solvent as in Example 1 was 0.44.

Example 5

1120 Micromoles of soy phosphatidylethanolamine were taken to dryness under high vacuum. 3360 Micromoles of monomethyl polyethylene oxide carboxylic derivative (average molecular weight 224) and 2240 micromoles of 1,1 carbonyl diimidazole were dissolved in 10 ml. of dry benzene and heated at 40°C until the bubbling had ceased. The solution was added to the dry phospholipid and the volume was reduced to 3 ml. The solution was heated for 3 hours at 70°C. The product was purified by silicic acid chromatography. The yield of the product was 53%. The R_f of the product in the same solvent system as in Example 1 was 0.61.

CLAIMS

1. A phosphatidyl alkylene oxide compound characterised by the structural formula:



where R_1 and R_2 are hydrogen or organic C_2-C_{25} acyl, R_3 is C_2-C_3 polymethylene, R_4 is C_2-C_{10} polymethylene, X is hydrogen or alkyl and n is a number from 0 to 200.

2. A compound according to claim 1 wherein R_1 and R_2 are C_8-C_{20} fatty acid groups.

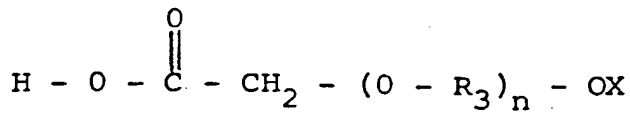
3. A compound according to claim 1 wherein R_1 and R_2 are acyl groups as found in soy phospholipids.

4. A compound according to claim 1 wherein R_1 and R_2 are acyl groups as found in egg phospholipids.

5. A compound according to any one of the preceding claims wherein n is from 3 to 20.

6. A compound according to any one of the preceding claims wherein R_4 is dimethylene.

7. A method of preparing a phosphatidyl polyalkylene oxide compound which method comprises coupling a phosphatidyl C_2-C_{10} alkanolamine to a carboxylic analog of a polyalkylene oxide polymer having the formula:



where R_3 is C_2-C_3 polymethylene and X is hydrogen or alkyl and n is a number from 0 to 200.

8. A method according to claim 7 which includes purifying the resulting coupled phospholipid compound by column chromatography.

9. A method according to claim 7 or 8 wherein the carboxylic analog has been obtained by oxidizing a C_2-C_3 polyalkylene oxide polymer.

10. A method according to claim 7, 8 or 9 wherein the coupling is carried out in a dry inert organic solvent.

11. Use of a phosphatidyl alkylene oxide as claimed in any one of claims 1 to 6 as an encapsulating agent in a drug-delivery system.

European Patent Office

EUROPEAN SEARCH REPORT

0072111
Application number

EP 82 30 3789.0

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	DE - B2 - 2 601 207 (TANABE SEIYAKU) & US - A - 4 016 100 --	11	C 07 F 9/10 C 08 G 65/32 A 61 K 9/66
A	GB - A - 2 051 069 (NATTERMANN) --		
D,A	US - A - 3 085 100 (S.S. CHANG et al.) --		
A,P	Chemical Abstracts vol. 96, no. 16 19 April 1982 Columbus, Ohio, USA M. SZOGYI et al. "Study of compounds affecting membrane function using artificial membrane" page 462, column 2, abstract no. 129747k & Magy. Tud. Akad. Biol. Tud. Oszt. Kozl., vol. 24, nos. 1-2, 1981 pages 201 to 207 --		TECHNICAL FIELDS SEARCHED (Int.Cl. 3) A 61 K 9/66 C 07 F 9/10
D,A	US - A - 2 310 679 (M. DE GROOTE et al.) --		
D,A	US - A - 4 145 410 (B.D. SEARS) ----		CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same category
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document

X The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Berlin	02-11-1982	KAPTEYN

EP Form 1503.1 06.78